In Claim 40, claim line 1, delete "36" and substitute therefor --37--.

In Claim 41, claim line 1, delete "36" and substitute therefor --37--.

In Claim 42, claim line 1, delete "40" and substitute therefor --41--.

In Claim 43, claim line 1, delete "40" and substitute therefor --41--.

REMARKS

Claims 1-42 are pending in the application. Support for the amendments are as follows. The amendment to the specification replaces "Bovine HLA" with "Bovine Leukocyte Antigen". This amendment corrects a clear typographical error. HLA stands for human leukocyte antigen, which is clearly not an antigen encoded by bovine genes. The genes being analyzed are the bovine leukocyte antigen genes, abbreviated BoLA.

In the claims, the newly added claims are renumbered. In addition the claim dependencies have been changed to reflect the change in the numbers of the claims. Therefore, no new matter is added by any of the amendments.

Rejections under 35 U.S.C. §112, First Paragraph

At the close of prosecution of the parent application, the specification was objected to and Claims 1-16 were rejected under 35 USC 112, first paragraph, for lack of enablement. Applicant's Attorney notes that no art-based rejections of those claims remained. The Examiner stated that Claims 1-16 are broad and encompass all multi-allelic loci and that the exemplification in the specification did not provide sufficient assurance that the method was applicable to genetic loci

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generally. However, the Examiner found that the method was enabled for analysis of HLA loci.

Therefore, it was determined in the parent application that the inventive method is applicable to all HLA loci for the analysis of alleles and haplotypes. The record also clearly indicates that the enablement rejection was based on the amount of exemplification in the Specification and the Examiners' need to be confident that the method would also be useful for multi-allelic loci outside of the HLA genes and related gene families. That is, the enablement rejection was based on the concern that the method may not be applicable to loci generally, rather than that one of ordinary skill would not understand how to apply the method to other loci.

Accompanying the previous amendment was a Declaration by Professor Peter Gresshoff, one of the Declarants in the parent application. Recent data obtained by Professor Gresshoff demonstrates that the method is also applicable to analysis of the soybean supernodulation gene locus. This data convinced Professor Gresshoff that the method applies to all eukaryotic genomes.

More specifically, Professor Gresshoff stated that when he signed his first Declaration, he felt that the phenomenon observed by Dr. Simons (that non-coding region polymorphisms were indicative of coding region polymorphisms and thus coding region alleles) probably related to genes generally. However, because the data was in the HLA genes, there was a question in his mind as to whether this phenomenon could be limited to members of the immunoglobulin super-gene family or to gene families with high coding region variability due to some characteristics common to such genes. However, now he found that soybean supernodulation gene region exhibits the same kind of polymorphism. More specifically, Professor Gresshoff found

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sequence heterogeneity in a one kilobase intergenic region where restriction endonuclease digestion revealed no differences in the sequences of the region. He also determined that some of these non-coding region polymorphisms were indicative of the soybean cultivar from which the DNA sample was taken.

This data convinced Professor Gresshoff that the phenomenon that non-coding region sequences contain informative polymorphisms that can be used to identify associated coding region alleles is a general phenomenon. Professor Gresshoff explicitly stated that he believes that the "the basis for Malcolm Simons' analysis system applies to all eukaryotic genomes."

Professor Gresshoff explained that he found these additional data convincing for a number of reasons. The first is that Professor Gresshoff was simply working in a non-coding region of the soybean genome and found this pattern of variation. That is, he did not design an experiment to verify Dr. Simons discovery. In addition, Professor Gresshoff believes that the NTS gene is a conserved gene. Because the NTS gene is conserved, no mechanism related to highly polymorphic loci should be present in the NTS gene. Clearly, the finding in the NTS gene also indicates that the phenomenon is not limited to the HLA genes or the immunoglobulin supergene family. Despite the vast differences between a conserved plant gene and the HLA genes, Professor Gresshoff observed the same correlation of non-coding region polymorphisms with coding region polymorphisms which is present in the HLA genes in the soybean NTS gene. In addition to demonstrations of this non-coding region micro-heterogeneity in both highly polymorphic and conserved genes, the data were obtained in humans and in soybeans. Furthermore, Professor Gresshoff's data were found in intergenic regions which are clearly more distant from the

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variable exon than the intervening sequences of the HLA genes described in the Specification. Clearly, this indicates that the phenomenon is not limited to humans or even animals, to HLA or the immunoglobulin supergene family, or to highly variable genes.

Professor Gresshoff concludes that since the analysis method is applicable to both the HLA loci in humans and the supernodulation locus in soybean, the approach is applicable to eukaryotic genomes generally. As established in the parent application, Professor Gresshoff is an expert in basic genetics, specializing in plant molecular genetics. His opinion is based on facts and he has provided a clear explanation as to why the facts are convincing. Applicant's Attorney notes that it would be difficult to find a DNA sequence much less similar to the HLA gene sequences. No further demonstration of the general applicability of the method should be required. Professor Gresshoff's expert opinion is sufficient to overcome the doubts of the Examiners in the parent application and to overcome the 35 U.S.C. §112, first paragraph rejection. Accordingly, withdrawal of the rejection is respectfully requested.

Art-based Rejections for Claims 1-16 and 35-43

Claims 1-16 are identical to Claims 1-16 in the parent application at the time the first Advisory Action was mailed. That Advisory Action indicated that all of the art based rejections of Claims 1-16 and 35-36 had been overcome. Since the 35 U.S.C. §112, first paragraph, rejection is now overcome, those claims are now allowable.

New Claims 37-43 were not considered in the parent application. However, the claims are distinct from the prior art in a manner similar to that of Claim 1-16 and 35-36. Newly added Claims 37-43 do not require amplification. However, the

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claims require that a coding region allele of a multi-allelic genetic locus is determined by identifying sequence polymorphisms characteristic of the alleles. The characteristic sequence polymorphisms are identified in a non-coding region sequence which is not more than about two kilobases in length.

As is clear from the record in the parent application, Applicant's method is based on his discovery that non-coding region sequences are informative and contain sequence polymorphisms that correlate with the coding region alleles and sub-allelic groups (e.g., haplotypes) of adjacent and remote genetic loci. Applicant has not invented a new way to analyze genetic loci. Rather Applicant has found that when prior art techniques are applied to the non-coding sequences, the result can be more informative than analysis of the coding regions. Furthermore, Applicant has found that analysis of relatively short regions of non-coding sequences, of a size which can be amplified, can provide more information than prior art analyses such as cDNA RFLP analyses which involve the use of significantly larger DNA sequences (20 to 40 or more kilobases).

As is clear from the record, the prior art analyses to determine either which allele of a gene is present in a sample or to determine which of two parental alleles is present in a sample (knowing only that one allele was "normal" and the other was disease-associated) could be either direct or indirect. Restriction sites are sites of polymorphism that can be used in family studies to distinguish chromosomes to attempt to identify whether the child is affected by a disease. The site can only be used to determine which of the maternal chromosomes is inherited if the mother is heterozygous for the marker. Since the mother is a carrier, rather than affected by the disease, the mother necessarily has two different alleles

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of the disease gene and is therefore a heterozygote. If the mother can be homozygous for the site, the site does not correlate with the alleles. The site is used to distinguish between the mother's chromosomes. These sites clearly were not used to identify a particular allele of the disease gene since the first criteria for use of the site was that it was polymorphic in the heterozygous mother.

Such analyses are described in the Saiki article (*Science* **230**:1350-1354 (1985), cited in the Information Disclosure Statement filed on December 21, 1992 and listed as Reference AT on the Form 1449, page 5) on DNA analysis methods used in prenatal diagnosis. The article states that both indirect and direct methods are used. Direct methods are said to be methods that do not require use of a family member and can be performed using only the DNA of the potentially affected child. Each of the direct methods actually detects a coding region sequence polymorphism characteristic of the coding region allele. For example, the sickle cell disease is caused by a point mutation of the β-globin gene that alters a restriction site in exon six of the gene. Analyses that amplify a region around the site of the mutation and then cleave the site with the enzyme can be used to determine whether the site, and therefore the mutation, is present in the gene sequence. Another direct method is probing or amplifying genomic DNA to determine whether the sample contains an α-globin gene, the absence of the gene causing α-thalassemia. Use of cDNA probes (which bind to coding region sequences) is also described.

The present invention relates to a direct analysis method in that the analyses do not require information regarding family members and can be performed using only the DNA to be analyzed. However, Claims 37-43 do not prevent one from using any prior art technique on a coding region sequence to detect a polymorphism in the coding region sequence, since the claims

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explicitly require the use of polymorphisms in the non-coding region sequence to identify coding region polymorphisms. Therefore, those direct methods which detect the polymorphism responsible for a disease or the absence of a gene that causes a disease neither anticipate nor make obvious the present claims.

The other type of analysis is indirect. As stated by Saiki these analyses involve the use of DNA from family members to attempt to determine whether the child has inherited a disease-associated gene. As clearly discussed in the Graham et al. (*Blood* 66:759-764 (1985), also cited on peviously filed PTO Form 1449 as Reference AS at p. 5) and Saiki articles, the family studies are based on the presence of a marker which is a site of polymorphism. The polymorphic site is either within the genetic locus to be analyzed or sufficiently close to the locus to be analyzed so that the locus and the marker are likely to be inherited together with a high degree of probability. The first requirement for use of such markers in a particular test is that the maternal (or paternal) DNA is heterozygous for the marker. (See the Graham article at page 761, column 1, second full paragraph.) Therefore, clearly the "marker" cannot be used to determine which allele of the locus is present. This is evident since the unaffected parents are necessarily heterozygous for the autosomal recessive disease gene and may or may not be heterozygous for the marker. That is, even if the marker is present in a non-coding region, the marker does not correlate with coding region alleles of the locus. As is clear from the claim language, the claims do not prevent anyone from identifying a marker which could be used as a site of polymorphism to determine inheritance in family studies.

Applicant's analyses do not use sites of polymorphism in non-coding regions to attempt to track inheritance of disease

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genes in family studies. Rather, Applicant's method is based on identifying sites of polymorphism in non-coding regions that indicate the allele or haplotype present in sample DNA. Prior art use of sites of polymorphism in inheritance pattern studies necessarily only identify the presence or absence of the marker which correlates with the transmission of the disease gene. Such determinations are not encompassed in the terms of the claims which require that the non-coding region polymorphism is characteristic of a coding region allele. Therefore, the pending claims do not preclude performing those prior art genetic analyses.

Similarly, the claims do not preclude other prior art uses of non-coding sequences. In addition to providing polymorphic sites, non-coding regions have been used in two general ways in genetic analyses. One is classical cDNA RFLP analyses. These analyses involve use of restriction endonucleases to cleave genomic DNA of an individual. No selection of any portion of the DNA prior to combination with an endonuclease is taught or suggested. Instead, all of the DNA of an individual is digested. Following endonuclease digestion, the resultant fragments are separated on a gel and blotted onto nitrocellulose. Those fragments associated with a genetic locus of interest are identified using a nucleic acid probe in a Southern blot analysis procedure. The probes are cDNA sequences that are specific to one (single locus) or more (multilocus) particular DNA sequences involved in the polymorphism.

Classical cDNA RFLP analyses can identify some alleles and haplotypes associated with a genetic locus. However, the cDNA RFLP patterns often cannot produce distinguishable patterns for all alleles or haplotypes associated with multi-allelic loci. Furthermore, as evidenced by the Erlich patent (U.S. Patent No. 4,582,788) and the Tenth International Workshop patterns of

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record in the parent application, the patterns involve the use of 20 to 40 or more kilobases of DNA.

In contrast, to the greater than 40 kilobases of DNA used to characterize some of the DQA1 alleles and haplotypes at the Tenth International Workshop, Applicant amplified 704 basepairs of DNA (249 basepairs of the exon and 527 of intron sequences, 438 of intervening sequence one and about 89 of intervening seqeunce two of DQA1) and digested the amplified DNA with restriction endonucleases. The DNA was from the same reference cell lines as used in the Tenth International Workshop. As demonstrated at the interview of February, 1992, both allele-specific and haplotype-specific patterns were identified. Furthermore, using Applicant's method the patterns were much more informative than the Tenth International Workshop patterns which used more than 50 times (40 kilobases in comparison to 0.7 kilobases) as large a DNA sequence.

Claims 37-43 are limited to use of a non-coding region sequence of a sufficiently small size (not more than about two kilobases in length), that the claims do not preclude use of prior art cDNA RFLP analyses. Furthermore, the striking difference in the amount of information provided by Applicant's method demonstrates that the prior art methods do not make the claims to the present method obvious.

Another way in which non-coding sequences were used in genetic analysis was the use of VNTR sequences as markers. However, rather than being used to mark the chromosomes having a disease gene, VNTR sequences were generally used in identity determinations in paternity testing and forensic applications. Specifically, a region of genomic DNA surrounding a VNTR sequence was amplified. Differences in the number of variable repeats result in length differences in the amplified sequence that can be used to distinguish the DNA of different individuals. VNTR sequences were not correlated to particular

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coding region alleles. Instead the VNTR sequences were used to determine either whether DNA from two sources (e.g., crime scene and suspect) were from the same individual or whether DNA from a putative father could comprise the obligate paternal contribution to DNA in a child. Therefore, like the prior art use of non-coding restriction sites as markers, prior art uses of VNTR sequences did not determine coding region alleles and are not encompassed by the claims. Those uses neither anticipate nor make obvious the claims.

Therefore, newly added Claims 37-43 clearly distinguish the prior art. Furthermore, Applicant's method represents a significant advance in being able to identify not only coding region alleles, but sub-allelic groups (haplotypes). Such information was only available using classical cDNA/RFLP analyses involving significantly longer regions of DNA. Allowance of the newly added claims is respectfully requested.

Rejection of Claims 17-27 and 33-34 under 35 U.S.C. §103

At the close of prosecution of the parent application, the Examiner stated that the art-based rejection of Claims 17-27 and 33-34 under 35 U.S.C. §103 had not been withdrawn. Those claims were rejected as obvious over Deng and Mullis in view of Erlich and Funke in the Office Action mailed July 29, 1991.

Claims 17-27 and 33-34 relate specifically to HLA analysis methods or to methods such as determination of paternity which are based on determination of HLA type. The Deng article teaches a method for PCR-RFLP analysis of DNA that distinguishes two alleles of the c-Ha-*ras* oncogene. An amplified sequence was produced that contains an *Hpa*II restriction site in the center of the sequence when the wild type allele is present and contains no *Hpa*II restriction site when the mutation is present. As stated by Deng, the *Hpa*II restriction site is in the 12th codon of the gene, not in an

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intron sequence. The article describes a particular assay for analysis of the ras oncogene. No way to apply the teachings of the Deng article to analysis of the HLA loci is taught or suggested by the article. The Deng method is only effective where the presence or absence of one RFLP site is associated with the presence or absence of a particular allele. No way to distinguish between a plurality of alleles is suggested by the method. The only way one could argue that the Deng article would be applicable to analysis of HLA loci was if there was a known restriction site that was associated with each of the alleles of an HLA loci. No such sites are described in any reference cited during the prosecution. Indeed, no such sites are reported. Furthermore, the use of such sites would only allow one to set up multiple RFLP digests, one for each allele associated with a locus. In contrast, the method of the present application provides patterns that characterize the alleles. The present method does not require one RFLP digest for each potential allele of the test locus. Therefore, Deng neither teaches nor suggests the method of Claims 17-27 and 33-34.

Mullis teaches the PCR method and that the method can be used to detect DNA polymorphism. Erlich teaching classical RFLP analysis of the HLA Class II region. The Examiner stated that the combination would make Claims 17-27 and 33-34 obvious. However, the RFLP analysis described by Erlich is not amenable to combination with the PCR methods of Mullis because the fragments analyzed in the Erlich reference are too large for amplification by the PCR method and sequence information surrounding the RFLP sites is not known. No reference which teaches or suggests that sufficient information to use a restriction digest of a DNA sequence of an amplifiable length was cited. Furthermore, the significantly larger sequences used in classical cDNA RFLP methods did not even provide the

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same amount of information as Applicant's method. This surprising ability to use a DNA sequence at least an order of magnitude smaller to obtain significantly more information is clearly an unexpected result which indicates that the claimed method is patentable. Allowance of Claims 17-27 and 33-34 is respectfully requested.

Lack of Double Patenting

Accompanying this Amendment is a Terminal Disclaimer, disclaiming the portion of any patent that issues on this application over the term of the patent that issues on the parent application (application Serial No. 07/551,239). Therefore, there is no question of obviousness-type double patenting.

The claims differ in scope from the allowed claims of the parent application, so there is no double patenting. Specifically, the allowed claims in the parent application corresponded to Claims 1-11 in this application, but were limited to analysis of HLA loci. The only pending claim of similar scope is Claim 12 which limits the analysis of Claim 12 to major histocompatibility (MHC) loci. Since the MHC loci include other species in addition to humans (encompassed in the term HLA loci) those claims differ in scope from the allowed claims in the parent application.

Since all of the outstanding rejections in the parent case have been overcome and the newly added claims distinguish the cited references in the same manner as the original claims of the application, it is believed that all of the claims are in condition for allowance.

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Early examination and allowance of this application is respectfully requested. If a telephone conference would expedite prosecution of the above-identified application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C., 20231, on January 14, 1993.

1/14/93
Date of Signature

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